FORM PTO-1390 (REV 12-2001) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: 45460-1

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 10/088532

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| PCT/CA00/01153 | October 3, 2000 | October 4, 1999 |

TITLE OF INVENTION
NON-SEPARATION HETEROGENOUS ASSAY FOR BIOLOGICAL SUBSTANCES

APPLICANT(S) FOR DO/EO/US
GAN, Zhibo

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ■ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Unsigned)
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11 to 20 below concern document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ■ Other items or information:
    1) International Preliminary Examination Report dated January 23, 2002
    2) Power of Attorney (unsigned)
    3) Credit Card Authorization Form

| U.S. APPLICATION NO. (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 10/088532 | PCT/CA00/01153 | 45460-1 |

| | CALCULATIONS PTO USE ONLY | |
|---|---|---|
| 21. ■ The following fees are submitted: | | |
| **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** | | |
| Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... **$1040.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... **$890.00** | | |
| International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... **$740.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... **$710.00** | | |
| International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... **$100.00** | | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | $ 890.00 | |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ■ 30 months from the earliest claimed priority date (37 CFR 1.492(e)). | $ 130.00 | |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | | |
|---|---|---|---|---|---|
| Total claims | 25 - 20 = | 5 | x **$18.00** | $ 90.00 | |
| Independent claims | 3 - 3 = | 0 | x **$84.00** | $ 0.00 | |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | 0 | + **$280.00** | $ 0.00 | |
| **TOTAL OF ABOVE CALCULATIONS =** | | | | $ 1,110.00 | |
| ■ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. + | | | | $ 555.00 | |
| **SUBTOTAL =** | | | | $ 555.00 | |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). | | | | $ | |
| **TOTAL NATIONAL FEE =** | | | | $ 555.00 | |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property + | | | | $ | |
| **TOTAL FEES ENCLOSED =** | | | | $ 555.00 | |
| | | | | **Amount to be refunded:** | $ |
| | | | | **charged:** | $ |

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. _____ . A duplicate copy of this sheet is enclosed.

d. ■ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

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NAME

28,584
REGISTRATION NUMBER

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Applicant : GAN, Zhibo

Serial No. :

Filed :

Title : NON-SEPARATION HETEROGENOUS ASSAY FOR BIOLOGICAL SUBSTANCES

Art Unit :

**KIRBY EADES GALE BAKER**
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The Hon. Commissioner of Patents
And Trademarks,
Washington, DC 20231 U.S.A.

Dear Sir:

**PRELIMINARY AMENDMENT**

As a Preliminary Amendment, please amend this application as follows.

**IN THE DISCLOSURE**

Between lines 1 and 2 of page 1 insert the following wording.

--CROSS-REFERENCE TO RELATED APPLICATION

This application is the national phase of international PCT application PCT/CA00/01153 filed October 3, 2000.

**REMARKS**

The reason for this amendment is to include a cross-reference to a related application.

Respectfully submitted,

Edwin J. Gale
Reg. No. 28,584
Our File No. 45460-1
April 2, 2002

# Non-separation heterogenous assay for biological substances

## TECHNICAL FIELD

The invention relates to the field of binding assays.

## BACKGROUND ART

Binding assays comprise a variety of methods that utilize the specific reaction between a ligand and corresponding binding agent such as protein. Immunoassay, receptor binding assays and lectin binding assay are examples of the binding assays. Immunoassay is by far the most common form which has been developed into an extremely versatile analytic technique with a diverse range of assay protocols.

The first quantitative precipitation test and purified antibody for the first time was developed by Heidelberger (1939). The practicability of this principle received wide attention and led to its exploitation in simple immunoassays that could measure single antigen systems. The diffusion of antibodies and antigen in agar gel (Oudin, 1946), immuno-electrophoresis (Grabar and Williams, 1953), antiglobulin test (Coombs, 1945), fluorescent labeled antibodies (Coons, 1941) etc has been used for the assay. A milestone in sensitive assays was radioisotopic labeling techniques for antibodies and antigens (Farr, 1958). The efforts formed the basis of the radioimmunoassay (RIA) which has been rapidly adapted by researchers and clinical laboratories. Nakane and Pierce (1966) demonstrated that enzyme could be coupled to antibody or antigen. The importance of this discovery is reflected in the now widespread application of chromogenic, fluorogenic, luminescent signals for the measurements with a similar sensitivity to that of RIA. These discoveries led to the

development of enzyme linked immunosorbent assay (ELISA) by Engvall and Perlmann (1971) which is widely used for researches and clinical tests at present.

Both RIA and ELISA involve the coating and separation of labeled and unlabeled antigen or antibody after the binding. These procedures inevitably use heterogenous phase and require separation of antigen and/or antibody unbound to the surface, this type of immunoassay, therefore, is called "heterogenous" immunoassay. Rubenstein et al (1972) developed a new immunoassay using an enzyme as a label in which the antigen-antibody reaction and its measurement are performed in solution without the need of prior separation of the free and antibody-bound components and without the use of solid phase. This type of separation-free immunoassay is called "homogenous" immunoassay.

Most enzyme assays are carried out for the purpose of estimating the amount or activity of an enzyme present in a cell, tissue, other preparation, or as an essential part of an investigation involving the purification of an enzyme. The current assay methods have been developed based on the physical, chemical and immunological properties where they can be detected using photometric, radiometric, high performance liquid chromatographic, electrochemical assays, etc. (Eisenthal, R. and Danson, M. J., 1993). Although the methods basically fulfill the many essential requirements for routine analysis, there are, among those, the varying disadvantages of low sensitivity (Brenda Oppert et al, 1997), multiple steps (Twining, S. S., 1994) and steps that are tedious and time-consuming (Fields, R., 1976). Immunoassays have been widely used in human clinical tests and therapeutics, agriculture, food, veterinary and environmental diagnostics. In most cases, immunoassays are effective and valid (Cleaveland, J. S. et al, 1990), but in some cases they are not suitable, for example, in the determination of enzyme activity. This occurs because the binding assays for antibody and antigen (enzyme) can only be used to measure the concentration of an antigen (enzyme) but not its activity. It is important to know the

catalytic activity of an enzyme and not just the amount of the enzyme as a given amount of the enzyme may have a widely varying activity depending on reaction conditions.

International PCT Application No. WO 99/32655 filed in the name of Nen Life Science Products, Inc. of Boston, Massachusetts, USA, published on July 1st, 1999, relates to a method for analyzing a sample for the presence and/or activity of an enzyme. The invention makes use of a hydrophobic layer formed on a solid support, the hydrophobic layer incorporating by hydrophobic interactions and amphipathic enzyme substrate labeled with a reporter on its hydrophilic region. The hydrophobic material having the substrate disposed therein is contacted with a sample and with a polar solvent, whereby enzyme present in the sample cleaves the substrate and produces a labeled hydrophilic fragment, which fragment migrates to the polar solvent. The presence of the reporter is then detected in the polar solvent or in the hydrophobic layer.

International PCT Application No. WO 98/16657 filed in the name of Chiron Corporation of Emeryville, California, USA, which was published on April 23rd, 1998, relates to a method of screening a compound capable of regulating protease activity by incubating a protease and a polypeptide in the presence and absence of a test compound. The polypeptide comprises an anchor region, a protease recognition site and a detectable signal region. The anchor region is bound to a solid support and the protease recognition site comprises (a) a protease cleavable peptide bond, and (b) at least three naturally adjacent amino acids on each side of the cleavable peptide bond. The signal region bound to the solid support is detected. Detecting an amount of the signal region bound to the solid support in the presence of the test compound, which is greater or smaller than an amount detected in the absence of the test compound, indicates that the test compound is capable of regulating protease activity.

It is important for developing a method to reduce the step of the measurement procedure.

## DISCLOSURE OF THE INVENTION

This present invention is for a method referred to as non-separation heterogeneous assay that greatly simplifies the detection, identification, measurement of concentration and activity of biological substances. It is based on the change of the label signal due to the distribution of the label between a solid surface and liquid in a vessel after completion of the reaction among reactants. The method involves the coating of a reactant (labeled or unlabeled) onto a surface, addition of a sample with or without a competitor labeled using a label tag or unlabelled. The change of the label signal can be directly measured.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a new method for the detection, identification, measurement of concentration and activity of biological substances, which is based on the change of the label signal due to the uneven distribution of the label between surface and liquid in a vessel after completion of the reaction among reactants.

1. An assay in which the surface of a vessel is coated with reactant 1 which is a binding ligand for reactant 3 being the labeled form of reactant 2. Competitive binding reactions between reactant 2 and reactant 3 for reactant 1 or between reactant

2 and reactant 1 for reactant 3 are initiated when an unknown amount of reactant 2 and a known amount of reactant 3 are added. The change of the label signal of the reactant 3 in the reaction vessel can be directly measured without an additional step and is directly proportional to the amount of reactant 2.

The reactions of the competitive binding between reactant 2 and reactant 3 for reactant 1 or between reactant 2 and reactant 1 for reactant 3 is interfered with by reactant 4 which is an inhibitor of reactant 2. The change of the label signal of the reactant 3 in the reaction vessel is directly proportional to the amount of reactant 4 (inhibitor).

2. An assay in which the surface of a vessel is coated with reactant 1 which is a labeled substrate for reactant 2 having a biological activity. The reaction is initiated when the reactant 2 is added. The change of the label signal in the reaction vessel can be directly measured and is proportional to the activity of reactant 2.

The reaction of hydrolyzation of reactant 1 coated on the surface of the vessel by reactant 2 is interfered with by reactant 3 which is an inhibitor of reactant 2. The change of the label signal is reciprocally proportional to the amount of reactant 3 (inhibitor).

## BEST MODES FOR CARRYING OUT THE INVENTION

## EXAMPLES

The following examples are for an illustrative purpose only, and not to limit the scope of the invention.

Example 1

The detection of DNA hybridization

Materials: Single stranded deoxyribonucleic acid (ssDNA), fluorescence labeled complementary ssDNA (fluo-cDNA), sample double stranded DNA (dsDNA) and a microplate.

Method:

1. Immobilization of ssDNA: ssDNA is added into the wells of a microplate containing spacer and incubated for the covalent end-linkage of DNA.

2. DNA assay:

(1). A series of concentrations of dsDNA in a buffer (50 ul/well) and sample are added to the wells of the microplate immobilized with ssDNA.

(2). A fixed amount of the fluo-cDNA (50 ul/well) is added to each well containing dsDNA and the control.

(3). Competitive hybridization between the fluo-cDNA and the dsDNA with the immobilized ssDNA are initiated by incubating at 85° C for a while and cool to room temperature for 1 h.

(4). The change of the fluorescent intensity of the fluo-cDNA in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of dsDNA.

Example 2

Competitive assay for an antigen

Materials: An antigen, fluorescence labeled antibody, sample containing the antigen and a microplate.

Method:

1. Coating of antigen: The antigen (100 ul/well) is added into the wells of a microplate and incubated at 37° C for 3 h. Then the microplate is washed 3 times using PBST.

2. Antigen assay:

(1). A series of concentrations of the antigen in a buffer (50 ul/well) are added to the wells of the coated microplate.

(2). A fixed amount of the fluo-antibody (50 ul/well) is added to each well containing the antigen and the control.

(3). Competitive binding reactions between the immobilized antigen and the free antigen (competitor) to the fluo-antibody are initiated by adding the fluo-antibody and incubated at 37° C for 2 h.

(4). The change of the fluorescent intensity of the fluo-antibody in the wells of the microplate is determined using a fluorometer and is directly proportional to the amount of the antigen in the sample.

Example 3

Fluorescent assay for protease and protease inhibitor

Materials: Protease, protease inhibitor, fluorescence labeled casein and a microplate.

Method:

1. Coating of a fluo-casein: fluo-casein (l00 ul/well) in PBS (pH 7.2) is added into the wells of a microplate and incubated at 37° C for 3 h and then the microplate is washed 3 times using PBST.

2. Protease activity assay:

A series of concentrations of a protease in a buffer (100 ul/well) and sample are added to the wells of the coated microplate and incubated at room temperature for 30 min. The change of the fluorescent intensity of the label in the wells of the microplate is measured with a fluorometer and is directly proportional to the activity of the protease.

3. Protease inhibitor assay:

(1). Varying amounts of the protease inhibitor in the buffer (50 ul/well) and sample are added to the wells of the coated microplate. Negative and positive controls are included.

(2). A fixed concentration of the protease in the buffer (50 ul/well) is added to the wells containing inhibitor and the controls and incubated at room temperature for 1 h. The change of the fluorescent intensity of the label in the wells of the microplate is measured with a fluorometer and is reciprocally proportional to the amount of the inhibitor.

REFERENCES CITED

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Fields, R. 1972 The rapid determination of amino groups with TNBS. Methods in Enzymology 25B: 464-468.

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Twining, S. S. 1994 Fluorescein isothrocyanate-labeled casein assay for proteolytic enzymes. Analytical Biochemistry 143: 30-34.

CLAIMS:

1. A competitive method for measuring the amount of a biological substance utilizing a competitive binding between the biological substances comprising:

a. the surface of a vessel coated with reactant 1.

b. a known amount of reactant 3 linked with a label and an unknown amount of reactant 2. The competitive reactions existing between reactant 2 and reactant 3 to bind to reactant 1 or between reactant 1 and reactant 2 to bind to reactant 3.

c. determining the change of the label signal of reactant 3 in the reaction vessel wherein the intensity of the label signal in the vessel is directly proportional to the amount of reactant 2.

2. The method of claim 1 wherein said reactant 1 is a receptor, said reactant 2 is a receptor binding ligand and said reactant 3 is the labeled form of reactant 2.

3. The method of claim 1 wherein said reactant 1 is a receptor binding ligand, reactant 2 is a receptor and said reactant 3 is the labeled form of reactant 2.

4. The method of claim 1 wherein said reactant 1 is a lectin, said reactant 2 is lectin binding ligand and said reactant 3 is the labeled form of reactant 2.

5. The method of claim 1 wherein said reactant 1 is a lectin binding ligand, said reactant 2 is a lectin and said reactant 3 is the labeled form of reactant 2.

6. The method of claim 1 wherein said reactant 1 is an enzyme, said reactant 2 is an inhibitor and said reactant 3 is the labeled form of reactant 2.

7. The method of claim 1 wherein said reactant 1 is an inhibitor, said reactant 2 is an enzyme and said reactant 3 is the labeled form of reactant 2.

8. The method of claim 1 wherein said reactant 1 is an antigen, said reactant 2 is an antibody and said reactant 3 is the labeled form of reactant 2.

9. The method of claim 1 wherein said reactant 1 is an antibody, reactant 2 is an antigen and said reactant 3 is the labeled form of reactant 2.

10. The method of claim 1 wherein said reactant 1 is a single stranded DNA (ssDNA), said reactant 2 is DNA containing complementary sequence of ssDNA and said reactant 3 is the labeled complementary ssDNA.

11. The method of claim 1 wherein said label is selected from the group of: fluorescent label, luminescent label, chromogenic label, and enzyme.

12. A method for detecting the biological activity of a biological substance utilizing the degradation of a substrate comprising:

a. the surface of a vessel coated with reactant 1 linked with a label.

b. addition of reactant 2 which has biological activity into the reaction vessel, said reactant 1 being hydrolyzed due to the activity of said reactant 2.

c. measuring the intensity of the label signal in the reaction vessel, wherein the change of the label signal in the vessel is directly proportional to the biological activity of said reactant 2.

13. The method of claim 12 wherein said reactant 2 is an enzyme and said reactant 1 is a substrate for the enzyme.

14. The method of claim 13 wherein said substrate is a polymeric or an oligomeric substrate.

15. The method of claim 13 wherein said enzyme is an enzyme that is able to cleave the substrate.

16. The method of claim 14 wherein said polymeric substrate is selected from the group of: carbohydrate, DNA, RNA, protein, PEG, or polypeptide.

17. The method of claim 14 wherein said oligomeric substrate is selected from the group of: peptide, oligosaccharide, or oligonucleotide.

18. The method of claim 13 wherein said enzyme is a protease or proteinase and said substrate is a protein.

19. The method of claim 13 wherein said enzyme is a carbohydrate hydrolase and said substrate is a carbohydrate.

20. The method of claim 13 wherein said enzyme is a DNase and said substrate is a DNA.

21. The method of claim 13 wherein said enzyme is a RNase and said substrate is a RNA.

22. The method of claim 13 wherein said enzyme is a peptidase and said substrate is a peptide.

23. The method of claim 13 wherein said enzyme is an oligosaccharide hydrolase and said substrate is an oligosaccharide.

24. The method of claim 12 wherein said label is selected from the group of: fluorescent label, luminescent label, chromogenic label, and enzyme.

25. A method for detecting the amount of an inhibitor to a biological substance comprising:

a. the surface of a vessel coated with reactant 1 linked with a label.

b. addition of a known amount of reactant 2 which has biological activity, an unknown amount of reactant 3 being an inhibitor of reactant 2 into the reaction vessel. The cleavage of reactant 1 by reactant 2 being inhibited due to the activity of reactant 3.

c. measuring the intensity of the label signal in the reaction vessel, wherein the change of the label signal in the vessel is reciprocally proportional to the amount of reactant 3.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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**Published:**
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

WO 01/25788 A1

(54) Title: NON-SEPARATION HETEROGENOUS ASSAY FOR BIOLOGICAL SUBSTANCES

(57) Abstract: This present invention is for a method referrred to as non-separation heterogenous assay that greatly simplifies the detection, identification, measurement of concentration and activity of biological substances. It is based on the change of the label signal due to the distribution of the label between a solid surface and liquid in a vessel after completion of the reaction among reactants. The method involves the coating of a reactant (labeled or unlabeled) onto a surface, addition of a sample with or without a competitor labeled using a label tag or unlabeled. The change of the label signal can be directly measured.

# DECLARATION AND POWER OF ATTORNEY

*As a below-named inventor, I hereby declare that:*

*My residence, post office address, and citizenship are as stated below next to my name.*

*I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:*

NON-SEPARATION HETEROGENEOUS ASSAY FOR BIOLOGICAL SUBSTANCES

*the specification of which (check one)*

____ *is attached hereto.*
xxx *was filed on* October 3, 2000 *as*
*Application Serial No.* PCT/CA00/01153

*and was amended on* April 2, 2002.
*(if applicable)*

*I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.*

*I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.*

*I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application on which priority is claimed:*

| *Prior Foreign Application(s)* | | | ***Priority Claimed*** | |
|---|---|---|---|---|
| ***Number*** | ***Country*** | ***Filing Date*** | ***Yes*** | ***No*** |
| 2,286,414 | Canada | October 4, 1999 | XXX | |
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*I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:*

| *Provisional Application No.* | *Filing Date* | *Status* |
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*I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application:*

| *Application Serial No.* | *Filing Date* | *Status* |
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***And I hereby appoint***
***Christopher C. Dunham (Reg. No. 22,031), Richard S. Milner (Reg. No. 33,970), Ivan S. Kavrukov (Reg. No. 25,161), Norman H. Zivin (Reg. No. 25,385), William E. Pelton (Reg. No. 25,702), John P. White (Reg. No. 28,678), Peter J. Phillips (Reg. No. 29,691), Robert D. Katz (Reg. No. 30,141), Paul Teng (Reg. No. 40,837), Richard F. Jaworski (Reg. No. 33,515)***

*and each of them, all c/o Cooper & Dunham of 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith, and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.*

*Please address all communications and direct all telephone calls, regarding this application*

*to* Richard S. Milner *Reg. No.* 33,970

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*I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.*

*Full name of sole or first joint inventor* Zhibo GAN

*Inventor's signature* Zhibo Gan

*Citizenship* Canada *Date of Signature* June 22, 2002

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